The use of ^{15}N as a biomarker for feed conversion efficiency (FCE) in pigs using blood and hair samples

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By

Dr. (Paul) Long Cheng

Email: long.cheng@unimelb.edu.au
Cellphone: 0481905929

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Australasian
Pork Research
Institute Ltd
APRIL

Executive Summary

Breeding and performance assessment for feed conversion efficiency (FCE; live weight gain/intake) is an important productive trait for a sustainable pig industry. Using biomarkers to predict FCE in pig production is one way to achieve performance assessment from production systems without the need for live measurements (e.g. individual animal intake), which are challenging to quantify in commercial production. However, currently there is limited biomarker available for producers to use to predict FCE in pigs. Based on previous work in sheep and cattle, $\Delta^{15}N$ is proposed as a novel biomarker to indicate FCE in pigs. The study aimed to 1) Establish relationships between FCE and multiple biomarkers in pig using blood and hair samples, 2) Study the animal tissue $\delta^{15}N$ (i.e., the ratio changes in naturally occurring isotope ^{15}N and ^{14}N) turnover rate from different animal tissue samples, and 3) Investigate the relative FCE performance of pigs from maternal *vs.* terminal genetic lines fed either high *vs.* low energy diets.

In this study involving 80 pigs fed for 56 days, the terminal genetic line and pigs fed the high energy diet had 5% and 15% higher FCE than the maternal genetic line and pigs fed the low energy diet, respectively. The analysis showed that blood, plasma and hair $\Delta^{15}N$ are potential biomarkers to differentiate pigs with differing FCE. As a single biomarker, blood $\Delta^{15}N$ had a 34% higher accuracy to predict **individual pig** FCE variation, compared with blood IGF-1. At a **group level**, blood and plasma $\Delta^{15}N$ explained 98% and 92% ($P < 0.05$) of the between group (n = 4) average variation in FCE, which can be used to confidently monitor pig group production FCE (e.g., compare different pig management strategies impact on FCE). The study showed the turnover rate of $\delta^{15}N$ in plasma was likely to be faster than $\delta^{15}N$ in blood, which indicates blood and plasma $\delta^{15}N$ may be used separately to predict long term *vs.* short term pig FCE changes. Furthermore, pigs fed the high energy diets had 13% higher live weight gain, 16% greater hot standard carcass weight and 38% higher carcass P2 than their counterparts fed the low energy diets.

In conclusion, under commercial production systems, a high energy diet is essential to maximise pig growth performance, carcass weight and FCE. The $\Delta^{15}N$ has the potential to differentiate pigs with differing FCE. Further refinement of the hair sampling technique may allow hair to be used as a non-invasive (alternative sample to invasive blood sample) sample to predict FCE by measuring its $\delta^{15}N$. Future studies involving a larger pig population managed under diverse environments are needed to conclude the industry application of $\Delta^{15}N$. Through such a study, cost: benefit can be determined, while pig health, meat quality and reproductive performance can also be quantified. The studies will provide a comprehensive understanding on how and when the $\Delta^{15}N$ can be used to support the monitoring and potential selection of pig FCE.

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1. Introduction

Breeding and performance assessment for feed conversion efficiency (FCE; live weight gain/intake) is an important productive trait for sustainable pig industry. Using biomarkers to predict FCE in pig production is one way to achieve performance assessment from production systems without the need for live measurements (e.g. individual animal intake), which are challenging to quantify in commercial production. However, currently there is limited biomarker available for producers to use to predict FCE in pigs. Previous work conducted in beef cattle (Wheadon et al., 2014) and in sheep (Cheng et al., 2015) showed naturally occurring nitrogen isotopes fractionation ($\delta^{15}N$; [(^{15}N /^{14}N) sample - (^{15}N /^{14}N) air] / [^{15}N /^{14}N] air × 1000) inversely correlated with FCE in ruminants. When assessing the change of isotopes in animals relative to feed ($\Delta^{15}N$; animal $\delta^{15}N$ - feed $\delta^{15}N$) between time points, ruminants that were more efficient recorded a smaller $\Delta^{15}N$ in plasma, blood and wool. Wheadon et al. (2014) and Cheng et al. (2015) concluded that $\Delta^{15}N$ has the potential to be used as a low-cost biomarker of FCE in cattle and sheep. Further, Cantalapiedra-Hijar et al. (2018) revealed that liver nitrogen metabolism is a major driver of $\Delta^{15}N$, while rumen nitrogen metabolism contributes little to $\Delta^{15}N$. Therefore, there may be a potential to use $\Delta^{15}N$ to indicate FCE in monogastric animals such as pigs. This study aimed to 1) Establish relationships between FCE and multiple biomarkers, including $\Delta^{15}N$ in pig using blood and hair samples, 2) Study the $\delta^{15}N$ turnover rate from different animal tissue samples, and 3) Investigate the relative FCE performance of pigs from divergent genetic lines and fed a high *vs.* low energy diet.

2. Methodology

Animal Ethics Approval

The study was undertaken under the authority of Rivalea (Australia) Pty Ltd Animal Ethics Committee (application No. 18P005C).

Experimental Design and Animal Management

Eighty 12-week-old male pigs (Large White x Landrace (PrimeGrow™ Genetics, Corowa, NSW)) weighing 43 ± 6.3 kg (mean ± SD) were used in this study. Two divergent genetic (G) lines and diets containing two levels of digestible energy (DE) were evaluated using a 2 × 2 factorial design. The four treatments were maternal G (MG) line + high DE diet (MGHE); MG line + low DE diet (MGLE); terminal G (TG) line + high DE diet (TGHE); and TG line + low DE diet (TGLE). Animals were housed individually in pens in the Grower Finisher Discovery Centre at Rivalea, Corowa, NSW and fresh water was offered *ad libitum*. Each treatment group had 20 pigs and they were fed individually for one week for diet adaptation followed by a measurement period. Following a period of one-week acclimation in the facility and being fed their respective grower treatment diets *ad libitum,* each pig was fed restrictively at 3 x Maintenance (Maintenance = 106 kcal Metabolisable Energy/kg × LW (kg) $^{0.75}$, NRC, 1998) for an 8-week measurement period. The measurement period comprised week 1 to week 4 on grower diet, and week 5 to week 8 on finisher diet (Table 1).

The pigs were sourced from Rivalea Australia Pty Ltd, Corowa, Australia for this study. Total of 40 MG pigs and 40 TG pigs were selected, representing different genetic lines with either a low, or high degree of selection for growth trait, respectively. Each group was then divided into two equal sub-groups balanced for live weight (LW) measured at the start of the adaptation period. One MG and TG group received HE diet, while another MG and TG group received LE diet. The diets were formulated as a pellet based on DE by Rivalea (Australia) Pty Ltd with a target of the grower diet to contain 15.5 MJ DE/kg as-

fed (HE groups) and 13.0 MJ DE/kg as-fed (LE groups), and the finisher diet to contain 14.5 MJ DE/kg as-fed (HE groups) and 12.2 MJ DE/kg as-fed (LE groups). All diets were formulated to the treatment energy levels using commercial ingredients (Appendix 1).

Animal Measurements

Individual pig feed intake was quantified weekly. All pigs were weighed and measured for body length (from base of the tail to the tip of the snout) at two weekly intervals throughout the study. The FCE was calculated using the equation: FCE (kg/kg) = LW gain (kg/day) ÷ intake (kg/day). Feed samples from each diet type were collected prior to the measurement period and stored at -20° C prior to chemical analysis. Blood samples were collected by venepuncture of the jugular vein from each pig on measurement day 0, 14, 28, 42, and 56. Blood was then sub-sampled into tubes to collect plasma by centrifugation at 2000 g for 5 mins at 5° C. Ten pigs per group were randomly selected to measure hair growth rate. A handful of hairs were plucked from the lower back, above the tail of each pig at the start and the end of the study. Three representative, undamaged hairs (with hair root) per pig from each sampling were selected under microscope and measured for length. The hair growth rate over 56 measurement days was calculated for each group as: the average hair length at the end of the study (mm) – the average hair length at the start of the study (mm). Based on the hair growth rate for each treatment group, 80 undamaged hair (with hair root) samples collected at the end of the study were cut accordingly to exclude hair from pre-measurement period. At the end of the study, hot standard carcass weight (Trim 1, AUSMEAT standard) and carcass P2 were measured through the commercial abattoir (Rivalea, Corowa, Australia).

Chemical Analysis

Diet samples were oven dried at 65° C to a constant weight to measure dry matter (DM) content. Diet samples were ground and scanned by calibrated near infrared spectroscopy (NIRS™ DS2500, FOSS, Denmark, located at Rivalea, Corowa, Australia) to predict chemical composition. To remove dirt contamination, each hair sample was soaked overnight in distilled water before it was cleaned in an ultrasonic cleaner for 3 minutes. The hair was then soaked in a sodium hydroxide solution for 2 hours before washing with distilled water. The cleaned sample was dried at 25° C oven for 1 hour prior to analysis. Dried feed and hair samples, liquid blood and plasma samples were analyzed for $\delta^{15}N$ using a Thermo Flash 2000 HT (elemental analyser) paired to a Thermo Delta V Advantage (mass spectrometer). The analysis also quantified total nitrogen content in the plasma. Plasma urea nitrogen and blood insulin like growth factor-1 (IGF-1) were analyzed using a Cobas Integra 400 plus (Roche, Switzerland) and ELISA kit (R&D Systems, Inc, Minneapolis, MN, USA), respectively.

Statistical Analysis

Statistical analysis was performed using Genstat Version 16 (VSN International Ltd, Hemel Hempstead, UK). Results were considered statistically significant at $P < 0.05$. Pig production performance, plasma, blood and hair parameters were analysed using two-way ANOVA, with main factors G and DE and their interaction term, based on the individual pig measurement.

To examine the relationships between biomarkers and individual pig FCE, three types of regression analyses were performed: 1) Simple linear regression analysis using individual pig data, 2) Simple linear regression analysis with groups, which used individual pig data while allowed four treatment groups to be included in the model, and 3) Multiple linear regression analysis using individual pig data to investigate the use of multiple biomarkers combination to predict FCE.

To examine the relationships between biomarkers and treatment group FCE, simple linear regression analysis was performed using average treatment data (n = 4).

3. Outcomes

Dietary treatments induced a larger difference in FCE than divergent genetic lines in this study. The TG line and HE diet fed pigs had 5% and 15% higher FCE than the MG line and LE diet-fed pigs, respectively (Table 2). This highlights that the energy content in the diet is a major driver of FCE (Patience et al., 2015). The difference in FCE was mainly derived from LW gain difference in this study, as feed intake remained the same across four treatments (Table 2). The higher FCE and LW gain observed for HE pigs compared with LE pigs are consistent with previous animal studies (Cottle, 2003; King et al., 2004; El-Sabagh et al., 2013). Pigs fed HE diets had a 16% higher hot standard carcass weight and 38% higher carcass P2 than their counterparts fed with LE diets (Table 2). The higher P2 measured in pigs offered the HE diets may reflect the conversion of excess energy into fat stores (Suster et al., 2004; DPIRD, 2017). There was a trend ($P<0.10$) for a main effect of genetic line on carcass P2. These genetic differences reflected the higher negative selection pressure on FCR (intake: LW gain) and carcass P2 in the T line genetics compared to the M line genetics.

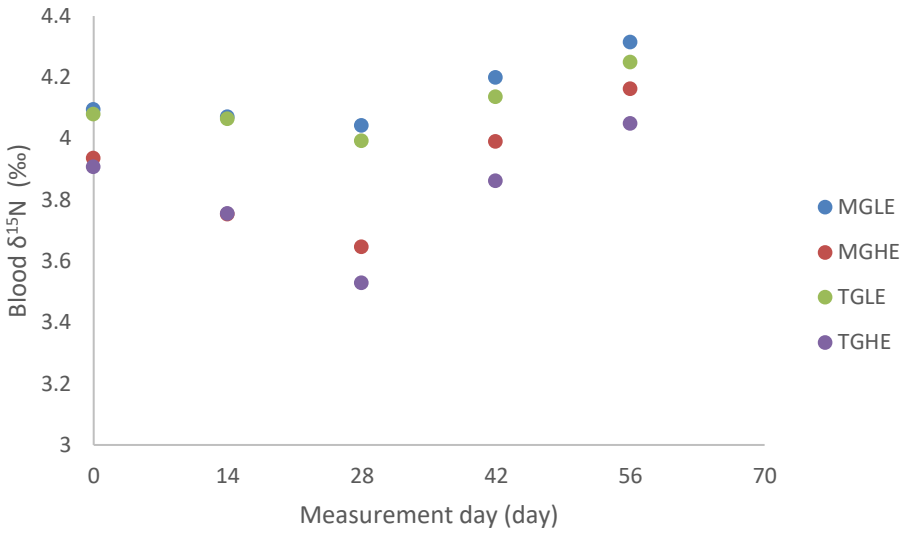
The switch from grower to the finisher diet on measurement day 28 provided a useful dataset to examine the relative turnover rate of the $\delta^{15}N$ in plasma and blood. Similar to Cheng et al. (2015), the current study showed that the turnover rate of $\delta^{15}N$ in plasma was likely to be faster than $\delta^{15}N$ in the blood. This was because across four treatments, the plasma $\delta^{15}N$ on average increased by 20%, while blood $\delta^{15}N$ increased by only 7% in response to the diet switch from grower to finisher diet on measurement day 28 (Figure 1). This important finding indicates blood and plasma $\delta^{15}N$ may be used separately to predict long term *vs.* short term pig FCE changes. However, a longer-term study is needed to confirm this speculation, as previous work showed it needs at least four weeks turnover time for blood to accumulate $\delta^{15}N$ and provide an adequate measurement of FCE in sheep (Cheng et al., 2015). The $\Delta^{15}N$ calculated from different average sampling date measurements for plasma $\delta^{15}N$ and blood $\delta^{15}N$ was explored to understand the best predictors from different time-points for FCE. The analysis revealed that taking plasma $\delta^{15}N$ average measurement on days 14, 28, 42 and 56, and blood $\delta^{15}N$ average measurement on days 28, 42 and 56 to calculate $\Delta^{15}N$, provided the best predictions of FCE in this study (Table 3).

As expected, simple linear regression analysis with groups showed that variation in FCE can be largely explained by the four designed treatments (Table 3). However, the major finding from this study was that the simple linear regression analysis (without group) showed that hair, plasma and blood $\Delta^{15}N$ had negative relationships with FCE, explaining 5, 19 and 43% ($P<0.05$) of individual pig FCE variation, respectively (Table 3, Figure 2). This result is in line with previous findings in cattle and sheep studies (Wheadon et al., 2014; Cheng et al., 2015) and it is likely linked to the nitrogen metabolism process (e.g. deamination, transamination) in the pig liver (Cantalapiedra-Hijar et al., 2018). Furthermore, it is interesting to note that $\Delta^{15}N$ measures explained more variation in FCE than the traditional FCE biomarkers tested in this study (i.e. plasma urea nitrogen and blood IGF-1 explained 0 and 10% of FCE variation, respectively) (Table 3, Figure 2). This is supported by the reported phenotypic correlations between IGF-1 and FCE in growing pigs ranged between 5% and 39% in an analysis, pooled data from 9 trials (Bunter et al., 2005). Comparable to the findings of using individual pig FCE data in this study, the use of average group plasma nitrogen, hair $\Delta^{15}N$, plasma $\Delta^{15}N$, blood $\Delta^{15}N$, and plasma IGF-1 explained 43, 10, 92, 98, and 69% variation in FCE between treatment groups (n = 4), respectively (Table 3). Low sample size (n = 4) tested in this study led to plasma nitrogen, blood IGF-1 and hair $\Delta^{15}N$'s association

with FCE being not statistically significant, but the overall results indicates that plasma $\Delta^{15}N$, blood $\Delta^{15}N$ have potential to be used to differentiate four groups on FCE differences, which is important to support producer decision making/management on farm. The use of plasma urea nitrogen was shown to have no potential for prediction of FCE within individual pigs (Table 3). Moreover, the multiple linear regression analysis showed that there was no improvement in FCE prediction by combining IGF-1 and $\Delta^{15}N$ in this study (no data presented). Future studies are needed to understand the potential to combine multiple biomarkers to enhance FCE prediction accuracy.

One objective of this study was to look at the potential of using a hair sample as a "non-invasive" method to predict FCE. In commercial production, it would be easier for a producer to collect a hair and send it for analysis, than rely on a qualified stockperson or veterinarian visit to take a blood sample. The findings presented in the section above highlighted the potential to use hair $\Delta^{15}N$ to predict FCE, which agrees with the results from Cheng et al. (2015), who showed a strong association between FCE and wool $\Delta^{15}N$ in sheep. However, at the pig group level, hair $\Delta^{15}N$ did not provide an accurate prediction to FCE in the current study. This inaccuracy in prediction may be related to the site and technique used to harvest hair samples. Further, assumptions made to calculate how much hair should be cut to exclude pre-measurement period hair may have been inappropriate since the calculation was done according to an average growth rate measured from 10 pigs per treatment group rather than measurement from the individual pig. Future studies are needed to refine the hair sampling technique, such as shaving off a patch of hair per pig and collect the regrowth of hair from the patch during the measurement period, which should better capture the $\delta^{15}N$ signature corresponding to the pig production performance change. This technique is often used in wool growth studies in sheep.

(A)



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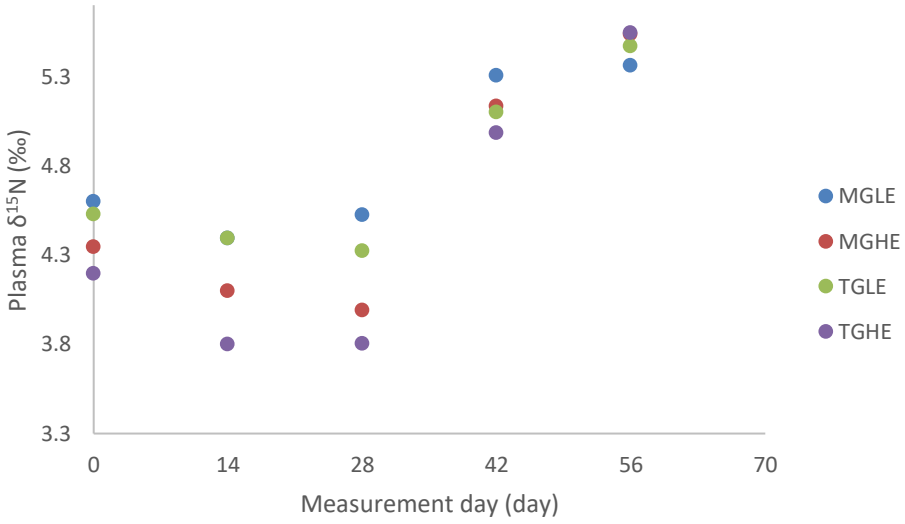


Figure 1. Blood **(A)** and plasma **(B)** $\delta^{15}N$ changes over time for pigs selected from maternal (M) *vs.* terminal (T) genetic (G) lines and fed with high (H) *vs.* low (L) energy (E) content diet (n = 20 per treatment group).

Table 1. Feed chemical composition of diets used in the experiment.*

	High energy content grower diet	Low energy content grower diet	High energy content finisher diet	Low energy content finisher diet
Crude Protein (%) (determined)	18.0	18.0	17.0	17.0
Crude Fat (%) (determined)	7.3	1.9	3.8	2.3
Digestible Energy (MJ/kg) (calculated)	15.5	13.0	14.5	12.2
Feed $\delta^{15}N$ (‰) (determined)	1.50	2.05	2.37	2.01

*For more details of feed formulation, please refer to Appendix 1.

Table 2. Intake, live weight (LW) gain, feed conversion efficiency (FCE), carcass characteristics, biomarkers measured in blood, plasma and hair of pigs selected from maternal (M) *vs.* terminal (T) genetic (G) lines and fed with high (H) *vs.* low (L) energy (E) content diet.

	MGHE	MGLE	TGHE	TGLE	*P* (G)	*P* (E)	*P* (G×E)	LSD[1]
Average 8 weeks intake (kg/day/pig)	2.39	2.41	2.33	2.40	0.422	0.279	0.527	0.078
Week 1-4 average intake (kg/day/pig) on grower diet	1.86	1.92	1.86	1.91	0.990	0.029	0.817	0.048
Week 5-8 average intake (kg/day/pig) on finisher diet	2.93	2.90	2.81	2.90	0.311	0.623	0.379	0.121
Average 8 weeks LW gain (kg/day/pig)	1.14	1.01	1.18	1.05	0.098	<0.001	0.870	0.044
Week 1-4 average LW gain (kg/day/pig) on grower diet	1.05	0.95	1.14	0.97	0.005	<0.001	0.046	0.039
Week 5-8 average LW gain (kg/day/pig) on finisher diet	1.23	1.08	1.22	1.13	0.498	<0.001	0.340	0.061
Average 8 weeks FCE (kg LW gain/kg intake)[2]	0.48	0.42	0.51	0.44	0.001	<0.001	0.273	0.014
Week 1-4 average FCE (kg LW gain/kg intake) on grower diet	0.57	0.50	0.62	0.51	0.001	<0.001	0.028	0.018
Week 5-8 average FCE (kg LW gain/kg intake) on finisher diet	0.42	0.37	0.44	0.39	0.022	<0.001	0.886	0.015
Body length change (cm/65 days)	29.1	26.7	30.3	28.2	0.106	0.008	0.825	1.65
Blood IGF-1 (ng/ml)[3]	401.6	333.8	389.1	330.9	0.615	<0.001	0.757	30.68
Plasma urea nitrogen (mmol/l)[4]	3.1	3.1	2.8	2.7	0.012	0.536	0.948	0.24
Plasma nitrogen (%)[5]	0.9	0.9	0.8	0.8	0.047	0.798	0.562	0.04
Blood $\Delta^{15}N$ (‰)[6]	2.0	2.2	1.9	2.1	0.007	<0.001	0.234	0.07
Plasma $\Delta^{15}N$ (‰)[7]	2.7	2.8	2.6	2.8	0.069	0.003	0.110	0.10
Hair $\Delta^{15}N$ (‰)[8]	3.9	4.0	3.6	3.7	0.142	0.506	0.925	0.36

Hot standard carcass weight (kg/pig)	80.9	67.5	83.7	73.8	0.027	<0.001	0.380	4.00
Carcass P2 (mm/pig)	14.1	9.6	12.2	9.4	0.064	<0.001	0.126	1.13

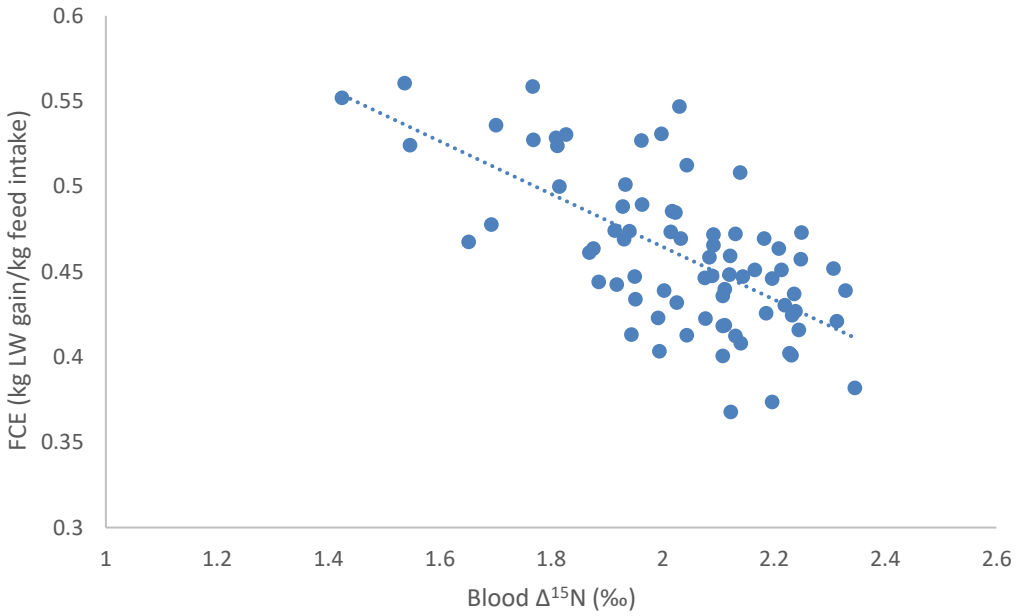
[1] LSD = Least significant difference $P < 0.05$.
[2] FCE = LW gain ÷ intake; measured between day 0 and 56.
[3] IGF-1= Insulin like growth factor-1; average measurement of days 14, 28, 42 and 56.
[4]Average plasma urea nitrogen measurement of days 14, 28, 42 and 56.
[5]Average plasma nitrogen measurement of days 14, 28, 42 and 56.
[6]Blood $\Delta^{15}N$ = blood $\delta^{15}N$ (average measurement of days 28, 42 and 56) – feed $\delta^{15}N$ (average measurement of grower and finisher diets).
[7]Plasma $\Delta^{15}N$ = plasma $\delta^{15}N$ (average measurement of days 14, 28, 42 and 56) – feed $\delta^{15}N$ (average measurement of grower and finisher diets).
[8]Hair $\Delta^{15}N$ = hair $\delta^{15}N$ – feed $\delta^{15}N$ (average measurement of grower and finisher diets).

Table 3. Correlations between feed conversion efficiency (FCE) and biomarkers measured in pigs selected from maternal *vs.* terminal genetic lines and fed with a high *vs.* low energy content diet.

	R^2	SE	*P*
Individual assessment (n=80)			
Blood IGF-1[1]	0.10	0.04	0.003
Plasma urea nitrogen[2]	0	0.05	0.76
Plasma nitrogen[3]	0.04	0.04	0.047
Blood $\Delta^{15}N$[4]	0.43	0.03	<0.001
Plasma $\Delta^{15}N$[5]	0.19	0.04	<0.001
Hair $\Delta^{15}N$[6]	0.05	0.04	0.028
Individual assessment (n=80 regression with groups*)*			
Blood IGF-1[1]	0.53	0.03	<0.001
Plasma urea nitrogen[2]	0.53	0.03	<0.001
Plasma nitrogen[3]	0.55	0.03	<0.001
Blood $\Delta^{15}N$[4]	0.63	0.03	<0.001
Plasma $\Delta^{15}N$[5]	0.57	0.03	<0.001
Hair $\Delta^{15}N$[6]	0.55	0.03	<0.001
Group Assessment (n=4)			
Blood IGF-1[1]	0.69	0.02	0.11
Plasma urea nitrogen[2]	0	0.05	0.95
Plasma nitrogen[3]	0.43	0.03	0.21
Blood $\Delta^{15}N$[4]	0.98	0.006	0.007
Plasma $\Delta^{15}N$[5]	0.92	0.01	0.028
Hair $\Delta^{15}N$[6]	0.10	0.04	0.37

[1]IGF-1= Insulin like growth factor-1; average measurement of days 14, 28, 42 and 56.
[2]Average plasma urea nitrogen measurement of days 14, 28, 42 and 56.
[3]Average plasma nitrogen measurement of days 14, 28, 42 and 56.
[4]Blood $\Delta^{15}N$ = blood $\delta^{15}N$ (average measurement of days 28, 42 and 56) – feed $\delta^{15}N$ (average measurement of grower and finisher diets).
[5]Plasma $\Delta^{15}N$ = plasma $\delta^{15}N$ (average measurement of days 14, 28, 42 and 56) – feed $\delta^{15}N$ (average measurement of grower and finisher diets).
[6]Hair $\Delta^{15}N$ = hair $\delta^{15}N$ – feed $\delta^{15}N$ (average measurement of grower and finisher diets).

(A)



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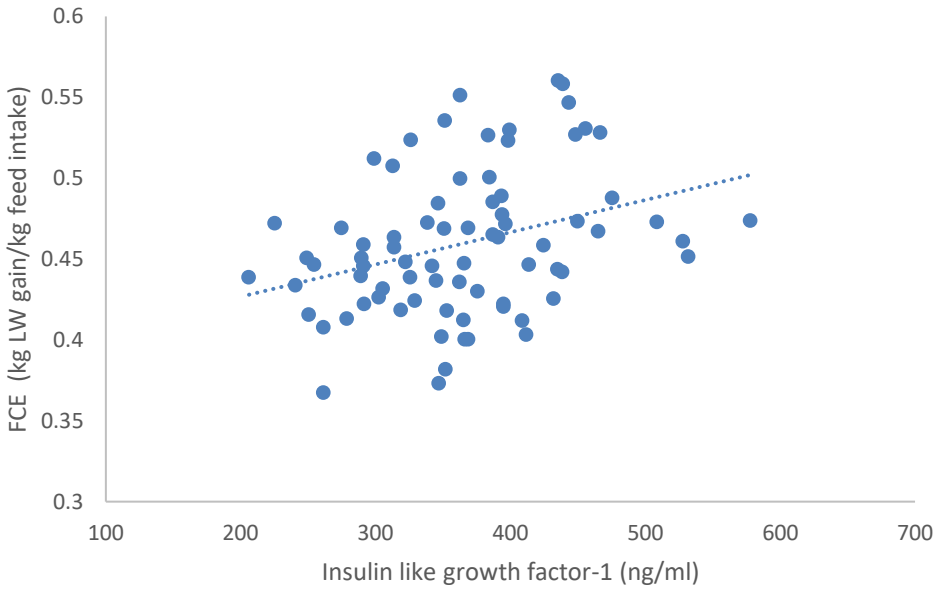

Figure 2. Use of blood $\Delta^{15}N$ (**A;** $Y = -0.15 \times X + 0.77$; $R^2 = 0.43$; SE = 0.03; $P < 0.001$) and IGF-1 (**B;** $Y = 0.0002 \times X + 0.387$; $R^2 = 0.10$; SE = 0.04; $P = 0.003$) to predict feed conversion efficiency (FCE) of pigs selected from maternal *vs.* terminal genetic lines and fed with high *vs.* low energy content diet.

4. Application of Research

- Blood and plasma $\Delta^{15}N$ have potential to monitor different pig production FCE and identify individual pigs with high FCE. The cost of $\Delta^{15}N$ is between $30-$50/sample, which should be acceptable to pig breeders and commercial producers.
- The potential for $\Delta^{15}N$ as a genetic trait will depend on how well the trait is genetically correlated with FCE. As this preliminary study did not assess this, further work is required to determine the use of $\Delta^{15}N$ for pig breeders.
- Plasma and blood samples should be taken, starting from week 2 and 4 of feeding, respectively. Multiple blood samples harvested at the different time-points can be pooled together prior to $\delta^{15}N$ analysis to reduce analytical cost.
- Further refinement of hair sampling technique may allow hair to be used as a non-invasive (alternative sample to invasive blood sample) sample to predict FCE by measuring its $\delta^{15}N$.

5. Conclusion

The study confirmed that there are potential biomarkers that could be used to detect genetic and/or dietary differences in feed efficiency of grower and finisher pigs with a reasonable degree of accuracy. However, the hair samples were not comparable to blood-derived biomarker predictability of FCE. The potential of using hair as an easy to harvest and non-invasive collection sample should be further explored with refined technique to harvest hair from pigs. For individual animals, $\Delta^{15}N$ does not appear to have the required level of accuracy to replace LW and feed intake recording of individual animal performance testing. The use of either a single biomarker or a combination of biomarkers for commercial estimation of feed efficiency in a group of pigs requires further investigation.

The study proved $\Delta^{15}N$ is a more accurate biomarker compared with IGF-1 to monitor different pig group FCE and identify individual pigs with high FCE. The potential for the $\Delta^{15}N$ as a genetic biomarker for feed efficiency is unknown and its suitability as a genetic selection marker will be dependent on the heritability of the $\Delta^{15}N$ trait.

6. Limitations/Risks

1. The current study only used a small population (n = 80) to preliminarily prove the usefulness of $\Delta^{15}N$ as a biomarker for FCE.
2. It is unknown the potential impacts of using $\Delta^{15}N$ to select FCE on pig health, meat quality and reproductive performance.
3. It is unknown how the commercial use of biomarkers in a group pen situation will be best implemented.
4. The practice of repetitive blood samples is a welfare risk and may need to be performed by qualified stockpersons or veterinarians, adding to the cost of the test.

7. Recommendations

Future studies involving larger pig populations managed under diverse environments are needed to conclude the industry application of $\Delta^{15}N$. Through such a study, cost: benefit can be determined, while pig health, meat quality and reproductive performance can also be quantified. This will provide a comprehensive understanding on how and when the $\Delta^{15}N$ can be used to support the monitoring and selection of pig FCE, and the number of samples required to achieve a similar degree of accuracy as measured FCE in group housed pigs.

8. References

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Appendix 1 -

	9571	9572	9573	9574
	18P005 GROW HI	18P0005 GROW LOW	18P005 FIN HI	18P005 FIN LOW
				Raw Material (%)
1 Wheat	72.316609	34.246666	78.510333	10.833333
12 Barley	.	25.5	.	35.465333
200 Milimix	.	20.0	.	35.0
300 Canola Meal 39%	.	.	6.0	6.0
325 Soybeanmeal-46%	15.366667	14.266667	7.633333	8.3
400 Meatmeal 60%	3.0	3.0	3.0	2.0
500 Water	1.0	1.0	1.0	1.0
504 Tallow-Enzyme	2.0	.	0.766667	.
520 Tallow-Mixer	4.1	.	1.5	.
560 Limestone	0.5	0.766667	0.7	0.933333
576 Dicalphos Bin Add	0.4	0.4	.	.
605 DL-Methionine	0.11	0.53333	0.03	.
627 Valine H/A	0.020057	.	.	.
1541 Phytase Micro	0.01	0.01	0.01	0.01
1544 Additive Micro	0.01	0.01	0.01	0.01
1551 Lysine Micro	0.546667	0.271667	0.343333	0.071667
1553 Threonine Micro	0.24	0.103333	0.133333	0.013333
1561 Copper Proteinate Micro 24%	0.041667	0.041667	0.033	0.033
1583 Tryptophan Micro	0.008333	.	.	.
1585 Salt Bin Micro	0.2	0.2	0.2	0.2
1592 All Spp Vit Blend A Micro Lar	0.025	0.025	0.025	0.025
1596 Mineral Ruminant Micro	0.031667	0.031667	0.031667	0.031667
1597 Mineral Monogastric Micro	0.04	0.04	0.04	0.04
1598 All Spp Vit Blend B Micro	0.033334	0.033334	0.033334	0.033334
	100.0	100.0	100.0	100.0
Nutrient	Analysis	Analysis	Analysis	Analysis
DE_PIG	15.502213	13.001359	14.505057	12.198345
NE4G	11.437977	9.166816	10.553559	8.491142
Protein	17.999098	18.000682	17.004899	17.007318
Fat	7.29405	1.909324	3.84986	2.295927
Fibre	2.369771	4.45495	3.096956	6.341371
Calcium	0.752965	0.849193	0.748048	0.753976
AV:PHOS	0.455043	0.502394	0.394054	0.431982
Alysine	1.085299	0.909814	0.841745	0.708358
#ALY/DE_	0.070009	0.069978	0.058031	0.05807
#MET/LYS	0.300647	0.300197	0.300684	0.30848
#M+C/LYS	0.548916	0.597179	0.630542	0.68754
#THR/LYS	0.670013	0.670405	0.700278	0.703707
#ISO/LYS	0.548471	0.639715	0.626499	0.721869
#TRY/LYS	0.182994	0.213812	0.209569	0.256001
#VAL/LYS	0.649689	0.788098	0.758952	0.952087